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FOR MORE INFORMATION
Lisa, M. Miller, Brookhaven National Laboratory, National Synchrotron Light Source, Bldg. 725D
lmiller@bnl.gov

Adele Qi Wang, Brookhaven National Laboratory, National Synchrotron Light Source, Bldg. 725D
qwang@bnl.gov

Co-Localization of β-Amyloid Deposits and Metal Accumulation in Alzheimer's Disease

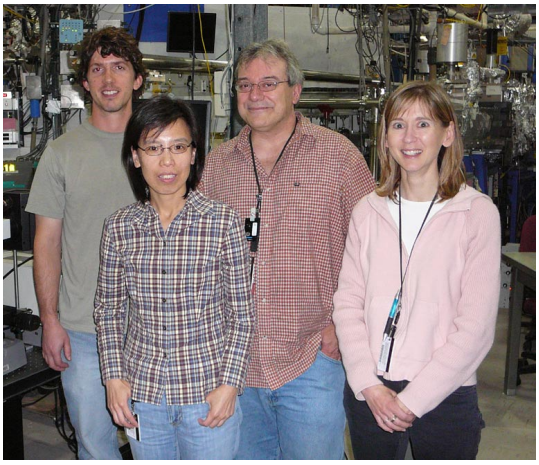
Q. Wang,[1] T. P. Telivala,[1] R. J. Smith,[1] A. Lanzirotti,[2] J. Miklossy[3], and L. M. Miller[1]

[1] National Synchrotron Light Source, Brookhaven National Laboratory; [2]Consortium for Advanced Radiation Sources, University of Chicago; [3]Kinsmen Laboratory of Neurological Research, University of British Columbia

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease. It is characterized by the misfolding and plaque-like accumulation of a naturally occurring protein, amyloid beta (Aβ) in the brain. This misfolding process has been associated with the binding of metal ions such as Fe, Cu, and Zn in vitro. In this work, the secondary structure of the amyloid plaques in human AD brain tissue was imaged in situ using synchrotron Fourier transform infrared microspectroscopy (FTIRM). The results were correlated spatially with the metal ion distribution in the identical tissue, as determined using synchrotron x-ray fluorescence (XRF) microprobe. Results revealed "hot spots" of accumulated Zn and Cu ions that were co-localized with the elevated regions of β-sheet protein, suggesting that metal ions may play a role in amyloid plaque formation in human Alzheimer's disease.

Alzheimer's disease (AD) is a progressive brain disorder that gradually destroys a person's memory, ability to learn, reason, make judgments, communicate, and carry out daily activities. The brain in AD is characterized by the presence of amyloid plaques, which consist of small deposits of a peptide called amyloid β (Aβ). *In vitro* evidence suggests that metal ions such as Cu, Zn, Fe and Mn may play a role in the misfolding of Aβ in AD. However, the functions of these metal ions and Aβ misfolding in the disease process are not well understood. The overall aim of this research is to obtain an *in situ* structural and mechanistic picture of how metal ions in the brain are involved in Aβ formation and plaque aggregation in AD.

In this work, thin cryosections (~ 10 μm) of brain tissue from patients with neuropathologically confirmed AD were studied. The locations of the amyloid plaques were visualized by green fluorescence using Thioflavin S staining and epifluorescence microscopy (**Figure 1B**). FTIRM carried out at NSLS beamline U10B showed that the amyloid plaques had elevated β-sheet content, as demonstrated by a strong Amide I absorbance at 1625 cm^{-1}, which was different from the FTIR spectrum of Aβ *in vitro* (**Figure 2A**). The correlation image generated based on peak height ratio of 1625 / 1657 cm^{-1} (**Figure 1C**) revealed that regions of elevated β-sheet content in the AD tissue corresponded well with amyloid deposits as identified by Thioflavin staining.



Authors (from left to right): Randy Smith, Adele Qi Wang, Antonio Lanzirotti and Lisa Miller.

Using XRF microprobe at NSLS beamline X26A and APS beamline 13-ID, we found that the background content of Ca, Fe, Cu, and Zn in AD *vs*. control tissue were similar; however the metal distribution in AD tissue was not uniform. Specifically, "hot spots" of accumulated Ca, Fe, Cu, and Zn ions were observed. The SXRF images of Zn and Cu can be seen in **Figure 1D and 1E**, respectively. A strong spatial correlation (r^2 = 0.97) was found between the locations of the Cu and Zn ions. The elevated Zn and Cu in the "hot spot" is also evident in representative XRF spectra (**Figure 2B**).

In order to correlate the misfolded amyloid protein and metal distribution in the tissue, a RGB image was generated with Zn content in red channel, β-sheet protein content in the green chan-

nel, and Cu content in the blue channel. Results revealed the co-localization of Cu, Zn and β-sheet protein in the amyloid plaques in AD human tissue (**Figure 1F**). Neither plaques nor accumulated metal hot spots were observed in control brain tissue.

In summary, these results provide increasing evidence that the formation of amyloid plaques from the Aβ peptide is associated with metal ions in the brain. Here we show for the first time a direct strong spatial correlation between AD plaques and metal ions in the brain, emphasizing the role of metal ions in AD etiology. In the future, exploring earlier stages of disease and *in-situ* probing metal-protein binding will be of interest for understanding the disease pathogenesis and mechanism.

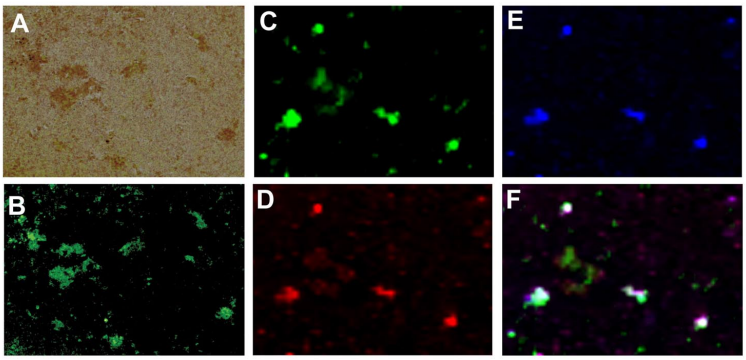


Figure 1. *(A) Bright field and (B) Epifluorescence image of human AD tissue stained with Thioflavin S. (C) Single channel color FTIR correlation image of β-sheet protein (green). (D) Single channel color SXRF microprobe image of Zn (red). (E) Single channel color SXRF microprobe image of Cu (blue). (F) The RGB correlation image.*

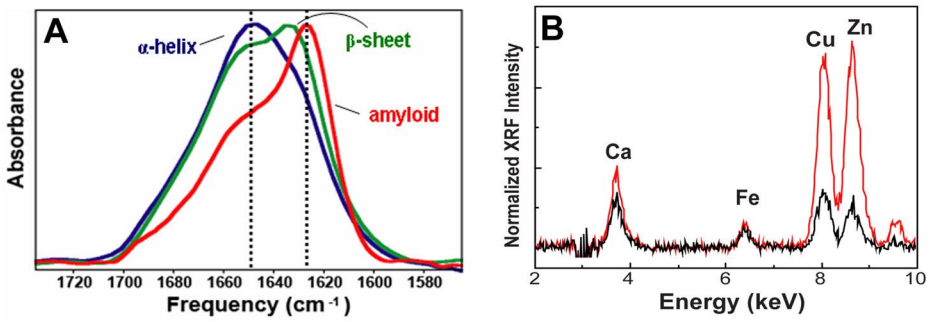


Figure 2. *(A) Infrared spectra of Thioflavin-positive area (red) and Thioflavin-negative area (black) of AD tissue. For comparison, the FTIR spectrum of purified Aβ peptide in vitro is shown (green). (B) SXRF microprobe spectra from Thioflavin-positive area (red) and Thioflavin-negative area (black).*